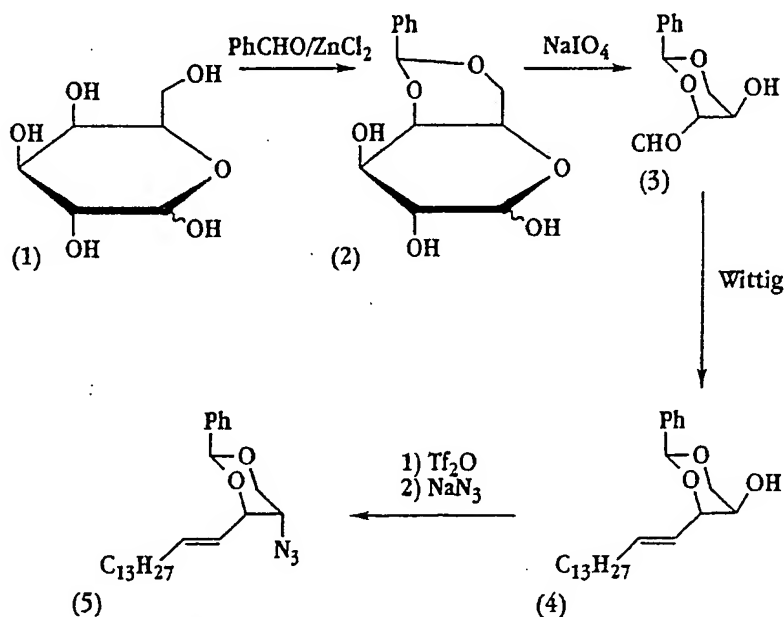




## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : C07H 9/04, C07C 213/00, 231/18		A1	(11) International Publication Number: WO 00/68238
			(43) International Publication Date: 16 November 2000 (16.11.00)
(21) International Application Number: PCT/IL00/00264		(81) Designated States: AU, CA, IL, JP, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).	
(22) International Filing Date: 9 May 2000 (09.05.00)			
(30) Priority Data: 60/133,526 10 May 1999 (10.05.99) US		Published With international search report.	
(71) Applicant: LIPIDERM LTD. [IL/IL]; Atarot Industrial Zone, P.O. Box 34048, 91340 Jerusalem (IL).			
(72) Inventor: ROCHLIN, Elimelech; Moshav Zanoach 67, D.N., 99888 Shimshon (IL).			
(74) Agent: REINHOLD COHN AND PARTNERS; P.O. Box 4060, 61040 Tel Aviv (IL).			

(54) Title: PROCESS FOR LARGE SCALE PREPARATION OF SPHINGOSINES AND CERAMIDES



(57) Abstract

Synthetic methods for convenient large scale preparation of D-erythro sphingosines and ceramides of high isomeric purity are described.

BEST AVAILABLE COPY

*FOR THE PURPOSES OF INFORMATION ONLY*

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

Process for Large Scale Preparation of Sphingosines and CeramidesField of the Invention

The present invention relates to synthetic methods for the preparation of D-erythro  
5 sphingosines and ceramides of high isomeric purity, and in particular to methods suitable for large  
scale production.

References

- Gros, E.G. and Deulofeu, V., *J. Org. Chem.* 29:3647-54 (1964).  
10 Herold, *Helv. Chim. Acta* 71:354 (1988).  
Kerscher, M. *et al.*, *Eur. J. Dermatol.* 1:39-43 (1991)  
Imokawa, G. *et al.*, *J. Soc. Cosmet. Chem.* 40:273-285 (1989).  
Kiso, M. *et al.*, *Carbohydrate Research* 158:101-111 (1986).  
Liotta, D. and Merrill, A.H., U.S. Patent No. 5,110,987 (1992).  
15 Polt *et al.*, *J. Org. Chem.* 57:5469 (1992).  
Schmidt, R.R. and Zimmerman, P., *Tetrahedron Lett.* 27(4):481-86 (1986).  
Schmidt, R.R. and Zimmerman, P., *Liebigs Ann. Chem.* 663-667 (1988).  
Schmidt, R.R. and Zimmerman, P., U.S. Patent No. 4,937,328 (1990).

Background of the Invention

Sphingosines constitute a group of related long-chain aliphatic 2-amino-1,3-diols, of which D-  
erythro-1,3-dihydroxy-2-amino-4,5-trans-octadecene is the most frequently occurring in animal  
tissues. N-acylsphingosines are also referred to as ceramides. Sphingosines, ceramides, and their  
glycosides, glycosphingolipids, are of great interest because of their diverse bioactivities and  
25 biological roles. These activities include inhibition of protein kinase C activity and transfer of  
information between developing vertebrate cells. Sphingosines also serve as chain terminators in  
various gangliosides. Galactosyl ceramide has been shown to be a receptor for HIV binding in cells  
lacking the CD4 receptor.

Skin ceramides are also believed to play an important role in the water permeability properties  
30 of the skin, providing an epidermal water barrier which strengthens the skin structure and reduces  
water loss. Ceramides and synthetic analogs have thus been used as components of skin care  
compositions, and have been found effective in restoring the water content of dry skin and in  
relieving atopic eczema (Kerscher *et al.*; Imokawa *et al.*).

These compounds have proven difficult to extract from natural sources, where they are present  
35 in low concentrations. Chemical synthetic methods reported to date have generally been laborious  
and expensive. Several reported methods of synthesizing optically pure sphingosines and their

derivatives rely on the use of serine as a chiral building block. See, for example, Polt *et al.*, Herold, and U.S. Patent No. 5,110,987. However, methods utilizing serine as a starting material are quite lengthy and thus are not amenable to potential scale-up. Other synthetic approaches to the preparation of isomerically pure sphingosines and ceramides have employed other chiral starting materials, such as carbohydrates, L-glyceric and D-tartaric acids, and/or asymmetric reactions. Although successful as gram-scale procedures, these strategies generally fail or become prohibitively expensive when applied to kilogram scale processes. Enzymatic methods of synthesis have also been described but are often unpredictable, giving varying results depending on medium or the particular enzyme preparation.

Accordingly, a reliable, convenient and versatile method of large scale preparation of these compounds is desirable.

### Summary of the Invention

The present invention includes, in one aspect, a convenient process for the large scale preparation of sphingosine, a sphingosine analog, or a ceramide. The process comprises the following series of steps. A stirred slurry of benzaldehyde and a Lewis acid, preferably  $\text{ZnCl}_2$ , is formed and contacted with D-galactose, with continued stirring. The resulting mixture is filtered to obtain a solid precipitate and a filtrate. The filtrate is then diluted with a mixture of diethyl ether and a hydrocarbon solvent, preferably a paraffinic solvent such as hexane, ligroin, or, more preferably, petroleum ether, typically in approximately equal proportions. The resulting mixture is extracted with cold water to provide an aqueous extract, which is treated with a base, such as an alkaline or alkaline earth carbonate or bicarbonate, to produce a precipitate of zinc salts. This precipitate is removed to provide an aqueous solution of 4,6-O-benzylidene-D-galactose. Alternatively, the zinc cation may be removed by treatment with an ion exchange resin. The resulting solution is then treated, preferably without isolation, with an oxidizing agent, preferably sodium periodate, which oxidatively cleaves the 4,6-O-benzylidene-D-galactose, to produce the protected hydroxy aldehyde, 2,4-O-benzylidene-D-threose. This compound is then contacted with a Wittig reagent, i.e.  $(\text{Ar})_2\text{P}=\text{CHR}$ , where Ar is aryl and R is a  $\text{C}_4$ - $\text{C}_{26}$  branched or unbranched alkyl or alkenyl chain, to produce a hydroxy olefin. Ar is typically phenyl but may also include alkyl substituted phenyl or naphthyl. The resulting compound is then reacted with a triflating agent, such as trifluoromethylsulfonic anhydride, followed by sodium azide, followed by a hydride reducing agent, such as  $\text{LiAlH}_4$  or  $\text{NaBH}_4$ , to produce an amino olefin. Finally, this compound is deprotected (i.e. the benzylidene group is removed) by contacting it with an acidic ion exchange resin, to produce sphingosine (where R is  $n\text{-C}_{13}\text{H}_{27}$ , tridecyl) or a sphingosine analog (where R is a longer or shorter alkyl chain, e.g.  $\text{C}_4$  -  $\text{C}_{12}$  or  $\text{C}_{14}$  -  $\text{C}_{26}$ ).

Alternatively, for the production of ceramides, the amino olefin is acylated, by treating with a

C<sub>2</sub> - C<sub>26</sub> acylating agent, such as an acyl halide, anhydride, or carboxylic acid; in the presence of any necessary acylating catalyst, prior to the deprotection step.

The invention also provides convenient large scale processes for the production of two of the intermediates, i.e. 4,6-O-benzylidene-D-galactose and 2,4-O-benzylidene-D-threose, by carrying out the process described above up to the oxidative cleavage step, or through the oxidative cleavage step, respectively. D-threose may also be obtained in large quantities by deprotection of the intermediate, 2,4-O-benzylidene-D-threose.

These and other objects and features of the invention will become more fully apparent when the following detailed description of the invention is read in conjunction with the accompanying drawings.

### Brief Description of the Drawings

Figure 1 shows a synthetic scheme for the large scale preparation of 1,3-O-benzylidene-2-azido-(D-erythro-sphingosine) (5) from D-galactose (1);

Figure 2 shows a synthetic scheme for the large scale preparation of sphingosine (7), dihydrosphingosine (14), and a ceramide (9) from the intermediate (5); and

Figure 3 shows a synthetic scheme for the large scale preparation of N-alkylated sphingosines.

### Detailed Description of the Invention

The process described herein, suitable for large scale production of sphingosines and ceramides, is based in part on the preparation of 1,3-O-benzylidene-2-azido-(D-erythro-sphingosine) (5) from D-galactose (1) as reported by Schmidt and Zimmerman (1986, 1988, 1990) and the sphingosine and ceramide preparations reported by Kiso *et al.* (1986). However, the present process incorporates significant modifications, resulting in substantial reductions in expenditure of time and labor. As used herein, "large scale" refers to kilogram, preferably multikilogram, quantities. The term "sphingosines" includes sphingosine itself and analogs which have the basic structure of sphingosine but vary in the length of the fatty alkyl chain.

As shown in Figures 1-3, the process may be used for the production of derivatives such as dihydrosphingosines, N,N-dimethyl- and N,N,N-trimethyl sphingosines, and ceramides having fatty acid components of various lengths. The process can also be modified for the preparation of phospho- and glycosphingolipids. The individual steps of the process will now be described.

#### A. Benzylidenation of D-galactose

The procedure followed by Schmidt and Zimmerman is based on that reported by Gros and Deulofeu (1964). The product, 4,6-O-benzylidene-D-galactose (BG) (2), was isolated from a reaction mixture initially containing benzaldehyde, ZnCl<sub>2</sub>, D-galactose, and 1,2,3,4-O-

digenzylidene-D-galactose. The product was isolated by a time- and labor-intensive procedure which included the following steps:

1. Decomposition of the reaction mixture in water,
2. Slow (overnight) separation of organic and aqueous phases at 5°C,
- 5 3. Washing of the organic phase with water,
4. Neutralization and  $\text{ZnCO}_3$  precipitation from the combined aqueous solution,
5. Filtration of  $\text{ZnCO}_3$  and washing of the filter with additional water,
6. Evaporation of the resulting aqueous solution to dryness *in vacuo* at ~40-45°C,
7. Extraction of the solid residue with boiling ethyl acetate to separate the 4,6-O-benzylidene
- 10 galactose (BG) from unreacted galactose and salts, and
8. Concentration of resulting ethyl acetate solution and crystallization of BG (2).

Stages of this procedure which are likely to be problematic for scaleup include the extended phase separation (step 2), during which the product is exposed to low pH, the extended evaporation (step 6), and the prolonged extraction of the solid residue with hot ethyl acetate (step 7). The quality of extraction can depend very much on the purity of the ethyl acetate. In some cases, the prolonged hot ethyl acetate exposure causes caramelization of galactose-BG-salt mixture and prevents complete extraction of the product.

In accordance with the present invention, the procedure was modified to overcome these problems, based in part on the following observations made by the inventors:

(1.) The undissolved residue remaining in the reaction mixture following the benzylidenation is not  $\text{ZnCl}_2$  as previously reported (Gros and Deulofeu), but unreacted D-galactose. When this residue is filtered off, no D-galactose is detected by TLC in the reaction mixture. Therefore, the unreacted D-galactose can be separated from the product by simple filtration of the reaction mixture prior to its decomposition by water, rather than by later extraction of the product with a hot solvent.

(2.) The phase separation after decomposition of the reaction mixture by water is accelerated by addition of an ether/hydrocarbon (preferably ether/petroleum ether) mixture.

(3.) After the aqueous phase is neutralized by  $\text{K}_2\text{CO}_3$  or  $(\text{Na}_2\text{CO}_3)$ , and  $\text{ZnCO}_3$  is filtered off (or, alternatively,  $\text{Zn}^{2+}$  cations are removed by ion-exchange resin IRC-50S), an aqueous solution of BG and salts (KCl or NaCl) is obtained. This aqueous solution is suitable for the further oxidation by  $\text{NaIO}_4$ ; it is not necessary to isolate the product (4,6-O-benzylidene-D-galactose).

An exemplary embodiment of this procedure is described in detail in Example 1, below. The process has also been carried out successfully using p-methoxybenzaldehyde in place of benzaldehyde.

#### B. Oxidative Cleavage of (2)

In the procedure of Schmidt and Zimmerman, isolated (2) was oxidized in aqueous or aqueous methanolic solution by addition of solid  $\text{NaIO}_4$  in the presence of phosphate buffer ( $\text{pH} = 7.0-7.6$ ).

The product, 2,4-O-benzylidene-D-threose (3), was isolated by evaporation of the resulting aqueous solution to dryness and extraction of the solid residue with THF, a very prolonged operation.

5 As stated above, in the present procedure, the aqueous solution of (2) from the previous step is oxidized directly, without isolation of (2). The solution thus obtained is concentrated to about one third of its initial volume and then extracted with dichloromethane ( $\text{CH}_2\text{Cl}_2$ ). Other organic solvents such as n-butanol or methyl ethyl ketone may also be used. The organic solution is rapidly dried by filtering through a short silica/ $\text{MgSO}_4$  (or silica/ $\text{Na}_2\text{SO}_4$ ) column. Evaporation of the  
10 solvent gave pure (3) as a white fragile foam that was easily broken up to a fine powder.

An alternative and possibly more convenient method of isolating (3) from the reaction mixture employs salting out with  $\text{Na}_2\text{SO}_4$  (rather than concentrating the solution) and then extracting with an organic solvent. This method would avoid prolonged concentration of the aqueous solution of (3), but larger amounts of solvent would be necessary in this case due to the high solubility of (3) even  
15 in concentrated brines.

An exemplary embodiment of the oxidation procedure is described in Example 2, below.

#### C. Wittig Alkenation of (3); Preparation of Azide (5)

For these steps, conventional procedures, such as described by Schmidt and Zimmerman, were  
20 generally suitable for kilogram scale reaction. The Wittig reaction gave a high *trans* selectivity (approx. 97% by NMR) and yielded about 4 kg of olefin (4) from 4 kg of (3). Wittig reagents having alkyl components of different lengths may be used to prepare various sphingosine analogs.

The triflation and azidolysis reactions were carried out according to known methods, giving  
25 1,3-O-benzylidene-azidosphingosine (5).

#### D. Deprotection Reactions

Schmidt *et al.* used azidosphingosine, prepared by  $\text{HCl}$ - or  $\text{pTsOH}$ -catalyzed deprotection of (5), as a key intermediate in the synthesis of sphingosine, ceramides, and other sphingolipids. These deprotection conditions, however, present disadvantages which become more pronounced in  
30 medium (ca. 50 g) to large scale (1 kg or more) production. The deprotection is incomplete even after extended reaction (12-78 hours) at room temperature, and the reaction mixture contains an appreciable amount of starting material and by-products. Heating the  $\text{CH}_2\text{Cl}_2/\text{MeOH}$  medium or employing  $\text{THF}/\text{HCl}$  leads to degradation of the product. (Although Schmidt *et al.*, 1986, reported that the method was "also successful in larger scale preparations", this observation referred to a  
35 20g scale synthesis.)

In addition, use of sphingosine (7) as a starting material in ceramide synthesis generally

requires protection of the OH groups or, alternatively, use of more selective and accordingly more expensive acylating agents. Otherwise, partial acylation of the OH groups occurs, requiring time consuming purification procedures.

For these reasons, 1,3-O-benzylidene sphingosine (6), prepared by reduction of (5) prior to  
5 deprotection, was used as the key intermediate in the present process (Figs. 2 and 3). It was synthesized by  $\text{NaBH}_4$  reduction of (5) in refluxing isopropanol (Fig. 2), as described in Example 3, below, or alternatively, by  $\text{LiAlH}_4$  reduction in ether at room temperature. This product was then used for the synthesis of D-erythro-sphingosine (7), ceramides (9), D-erythro-dihydrosphingosine (14), N,N-dimethylsphingosine (11), and N,N,N-trimethylsphingosine (13) (Figs. 2 and 3).

10 The procedure used for deprotection (Figs. 2 and 3) differed from that of Kiso *et al*, who used isopropylidene, rather than benzylidene, derivatives. Benzylidene derivatives give substantially better E/Z selectivity in Wittig alkenation than isopropenylidene derivatives. However, benzylidene deprotection under the conditions used by Kiso (acetic acid with small amounts of water) progresses very slowly at 5-50°C. At higher temperatures (60-80°C), the rate is still unsatisfactory and  
15 formation of by-products occurs.

Accordingly, more suitable procedures for deprotection of benzylidene derivatives (6), (8), (10), and (12), using ion exchange resins, were used for the present processes. Exemplary procedures, for conversion of (6) to sphingosine (7), and for deprotection of 1,3-O-benzylidene ceramides (8), are described below in Examples 4 and 5.

20 Figure 3 illustrates the N-alkylation of (6), followed by deprotection, to give N,N-dimethyl sphingosine (11) and the N,N,N-trimethyl ammonium salt (13). The alkylations were carried out using formaldehyde/sodium cyanoborohydride and dimethylsulfate/sodium bicarbonate, respectively. Preferably, alkylation is carried out prior to deprotection. As shown in Figure 2, catalytic hydrogenation of D-erythro sphingosine (7) gave D-erythro dihydrosphingosine (14); this  
25 reaction may be carried out before or after deprotection. All of the reactions illustrated in Figures 2-3 have been carried out successfully on a large scale.

The following examples illustrate but are not intended in any way to limit the invention.

## EXAMPLES

### 30 Example 1. Preparation of 4,6-O-benzylidene-D-galactose (2) (BG)

A portion of  $\text{ZnCl}_2$  (23 kg, 171 mol) was placed into a reactor, taking measures to exclude moisture. Benzaldehyde (70L, 680 mol) was poured into the reactor, and the mixture was stirred for about 30 minutes using a powerful mechanical stirrer. After initial dissolution of zinc chloride, a thick slurry of a benzaldehyde- $\text{ZnCl}_2$  complex forms. Anhydrous D-galactose (30 kg, 164 mol)  
35 and more benzaldehyde (60L, 570 mol) were added to the warm slurry, and the mixture was allowed to react at RT for 24 hours with vigorous stirring. The precipitate was filtered off and

washed portionwise with benzaldehyde (10-15L total) and then with acetone, to give, after air-drying, 6-7 kg of unreacted D-galactose, probably as the monohydrate. The combined filtrate and benzaldehyde washings were diluted with ether and petroleum ether, and the mixture was extracted with ice-cold water. The organic layer was washed 3 times with ice water, and the combined aqueous layers were neutralized by rapid addition of a solution of potassium carbonate. The resulting thick suspension of zinc salts was filtered, and the precipitate was washed thoroughly with water until 500-600 L of filtrate were collected. After extraction with chloroform (70L) and with petroleum ether (70L), the filtrate, containing 4,6-O-benzylidene-D-galactose (2) and a mixture of KCl and  $\text{KHCO}_3$ , and having a pH of about 8.7, was used directly for the following oxidation.

Example 2. Preparation of 2,4-O-benzylidene-D-threose (3)

The solution from Example 1 was placed into a reactor and buffered with a mixture of  $\text{K}_2\text{HPO}_4$  trihydrate (11 kg) and  $\text{KH}_2\text{PO}_4$  (4.5 kg). Sodium periodate (about 39 kg) was introduced into the solution by portions (approximately 0.5 kg) during 5-6 hours with vigorous stirring, maintaining the pH within 7.0-7.5 by periodic addition of 20% aq. KOH. During the addition, the temperature was kept at 20-25°C by external cooling.

The reaction suspension was concentrated *in vacuo* to approximately 150L. Dichloromethane (160L) was added, and the precipitate of inorganic salts was filtered off and washed thoroughly with  $\text{CH}_2\text{Cl}_2$  (additional 70L). The two-phase filtrate was allowed to separate, and the lower layer ( $\text{CH}_2\text{Cl}_2$  solution of (3)) was passed through a column (D = 30 cm) filled with dry  $\text{MgSO}_4$  (10 cm column), followed by silica gel (30 cm) and another layer of  $\text{MgSO}_4$  (10 cm). The aqueous layer was extracted 3 times with 70L portions of dichloromethane, and the extract was passed through the same column. The column was then eluted with 115 L chloroform to wash residual (3) from the silica gel. The combined eluates were concentrated *in vacuo* to a syrup and diluted with benzene to approximately 90 L. The solution was immediately dried *in vacuo* to give a solid foam. Yields of 2,4-O-benzylidene-D-threose (3) ranged from 12.5 kg to 13.6 kg (about 36-39% from D-galactose).

Example 3. Reduction of 1,3-O-benzylidene azidosphingosine (5)

Azido derivative (5) (100g, 0.25 mol) was reduced using 19 g (0.5 mol)  $\text{NaBH}_4$  in 1L of refluxing 2-propanol. The reaction mixture was worked up in the usual manner, filtered, and evaporated to dryness under reduced pressure. The solid residue was extracted with  $3 \times 500$  ml of boiling petroleum ether (60-80°) or hexane, and the combined extracts were filtered and evaporated. The solid residue was recrystallized from EtOH/ $\text{H}_2\text{O}$  to give 70 g (75%) of derivative (6): mp 51-52° C;  $[\alpha]_D +38.4^\circ$  (c = 0.6,  $\text{CHCl}_3$ ).

Example 4. Deprotection of 1,3-O-benzylidene-D-erythro-sphingosine (6)

A 50 g portion (0.129 mole) of 1,3-O-benzylidene-D-erythro-sphingosine (6) was dissolved in 1.5 L of 90% MeOH and passed through a column filled with an excess of a strongly acidic ion exchange resin. The column was then slowly eluted with 90% MeOH to elute benzaldehyde. The reaction is usually complete within 20-30 minutes at room temperature. The final product was then  
5 eluted with alkalized methanol, and dichloromethane and water were added to the eluate to give a two-phase mixture. The organic (lower) phase was separated, dried over  $\text{Na}_2\text{SO}_4$ , and evaporated to give ~ 36 g (94%) of 97-98% pure D-erythro-sphingosine, mp 73-76°C;  $^1\text{H}$  NMR spectrum in accordance with literature.

This process was also successfully applied to sphingosine analogs, and at a 5 kg scale, without  
10 any substantial deviations from the described procedure.

Example 5. Preparation and deprotection of 1,3-O-benzylidene-ceramides (8) with different fatty acid chains

Acylation of 1,3-O-benzylidene-D-erythro-sphingosine (6) was carried out using a variety of  
15 carboxylic acids ( $\text{C}_2$ - $\text{C}_{26}$ ), catalyzed by DCC, in  $\text{CH}_2\text{Cl}_2$ , or acid chlorides in  $\text{CH}_2\text{Cl}_2$ , according to known procedures. Yields of acyl derivatives (8) were 92-98%.

Protected ceramide (8) (~ 0.1 mol) was dissolved in 1.5-3.0 L (depending on the solubility of the ceramide) acetic acid at 60-70°C. Then ~ 200 g of strongly acidic ion exchange resin were added with stirring, followed by aqueous MeOH. The reaction mixture was stirred at 60-70°C until  
20 deprotection was complete by TLC and filtered. The filter was washed with hot acetic acid, and the combined filtrate was allowed to stand overnight at 0-4°C. The precipitate was filtered off, washed with 200 mL cold acetic acid, 1.5-2.0 L water, 500 mL satd.  $\text{NaHCO}_3$ , and 1-1.5 L water. After lyophilization, 70-85% yield of ~95% pure material was obtained. For higher purity, the precipitate was dissolved (without lyophilization) in  $\text{CHCl}_3$  or  $\text{CH}_2\text{Cl}_2$ , dried with  $\text{Na}_2\text{SO}_4$  and  
25 purified by column chromatography (1-5% MeOH/  $\text{CH}_2\text{Cl}_2$ ), giving > 99% pure ceramide (9).

Example 6. Preparation of D-erythro-dihydrosphingosine

A 0.67 mole portion of sphingosine or a sphingosine analog, prepared by the above methods, was dissolved in 3 L of absolute methanol, and 10 g of 5% Pd/C were added. Hydrogenation was  
30 carried out at room temperature under 1 atm pressure of  $\text{H}_2$ . The reaction mixture was filtered and concentrated by evaporation, and the residue was recrystallized from n-hexane, giving the product in 80-86% yield with 96-98% purity.

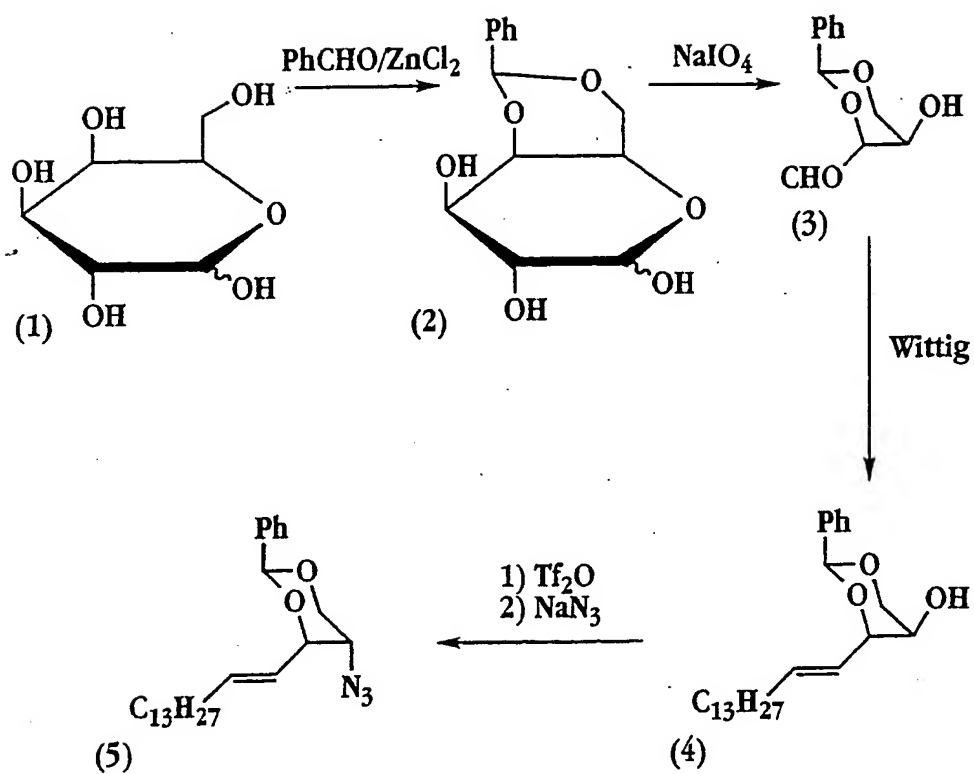
While the invention has been described with reference to specific methods and embodiments, it  
35 will be appreciated that various modifications may be made without departing from the invention.

## IT IS CLAIMED:

1. A process for the large scale preparation of sphingosine, a sphingosine analog, or a ceramide, comprising the steps of:
  - 5 forming a stirred slurry of benzaldehyde and  $\text{ZnCl}_2$ ,
  - contacting the stirred slurry with D-galactose,
  - filtering the resulting mixture to obtain a solid precipitate and a filtrate,
  - diluting the filtrate with diethyl ether and a hydrocarbon solvent,
  - extracting the resulting mixture with cold water to provide an aqueous extract,
  - 10 removing  $\text{Zn}^{+2}$  from the aqueous extract by treatment with base or an ion exchange resin, to provide an aqueous solution of 4,6-O-benzylidene-D-galactose,
  - treating the solution with an oxidizing agent effective to oxidatively cleave said galactose, to produce a hydroxy aldehyde,
  - contacting the hydroxy aldehyde with a reagent of the form  $(\text{Ar})_2\text{P}=\text{CHR}$ , where Ar is aryl
  - 15 and R is a  $\text{C}_2\text{-C}_{26}$  alkyl chain, to produce a hydroxy olefin,
  - reacting the hydroxy olefin with a triflating agent, followed by sodium azide, followed by a hydride reducing agent, to produce an amino olefin, and
  - deprotecting the amino olefin by contacting the amino olefin with an acidic ion exchange resin, to produce sphingosine or a sphingosine analog.
  - 20
2. The process of claim 1, for the large scale preparation of a ceramide, further comprising the step of acylating said amino olefin prior to said deprotecting step.
3. The process of claim 2, wherein said acylating employs a  $\text{C}_2 - \text{C}_{26}$  acylating agent.
- 25
4. A process for the large scale preparation of 4,6-O-benzylidene-D-galactose, comprising the steps of:
  - forming a stirred slurry of benzaldehyde and  $\text{ZnCl}_2$ ,
  - contacting the stirred slurry with D-galactose,
  - 30 filtering the resulting mixture to obtain a solid precipitate and a filtrate,
  - diluting the filtrate with diethyl ether and a hydrocarbon solvent,
  - extracting the resulting mixture with cold water to provide an aqueous extract, and
  - removing  $\text{Zn}^{+2}$  from the aqueous extract by treatment with base or an ion exchange resin, to provide an aqueous solution of 4,6-O-benzylidene-D-galactose.
  - 35
5. A process for the large scale preparation of 2,4-O-benzylidene-D-threose, comprising the steps of:

- forming a stirred slurry of benzaldehyde and  $\text{ZnCl}_2$ ,  
contacting the stirred slurry with D-galactose,  
filtering the resulting mixture to obtain a solid precipitate and a filtrate,  
diluting the filtrate with diethyl ether and a hydrocarbon solvent,  
5 extracting the resulting mixture with cold water to provide an aqueous extract,  
removing  $\text{Zn}^{+2}$  from the aqueous extract by treatment with base or an ion exchange resin, to  
provide an aqueous solution of 4,6-O-benzylidene-D-galactose, and  
treating the solution with an oxidizing agent effective to oxidatively cleave said galactose.

1/3

**Fig. 1**

2/3

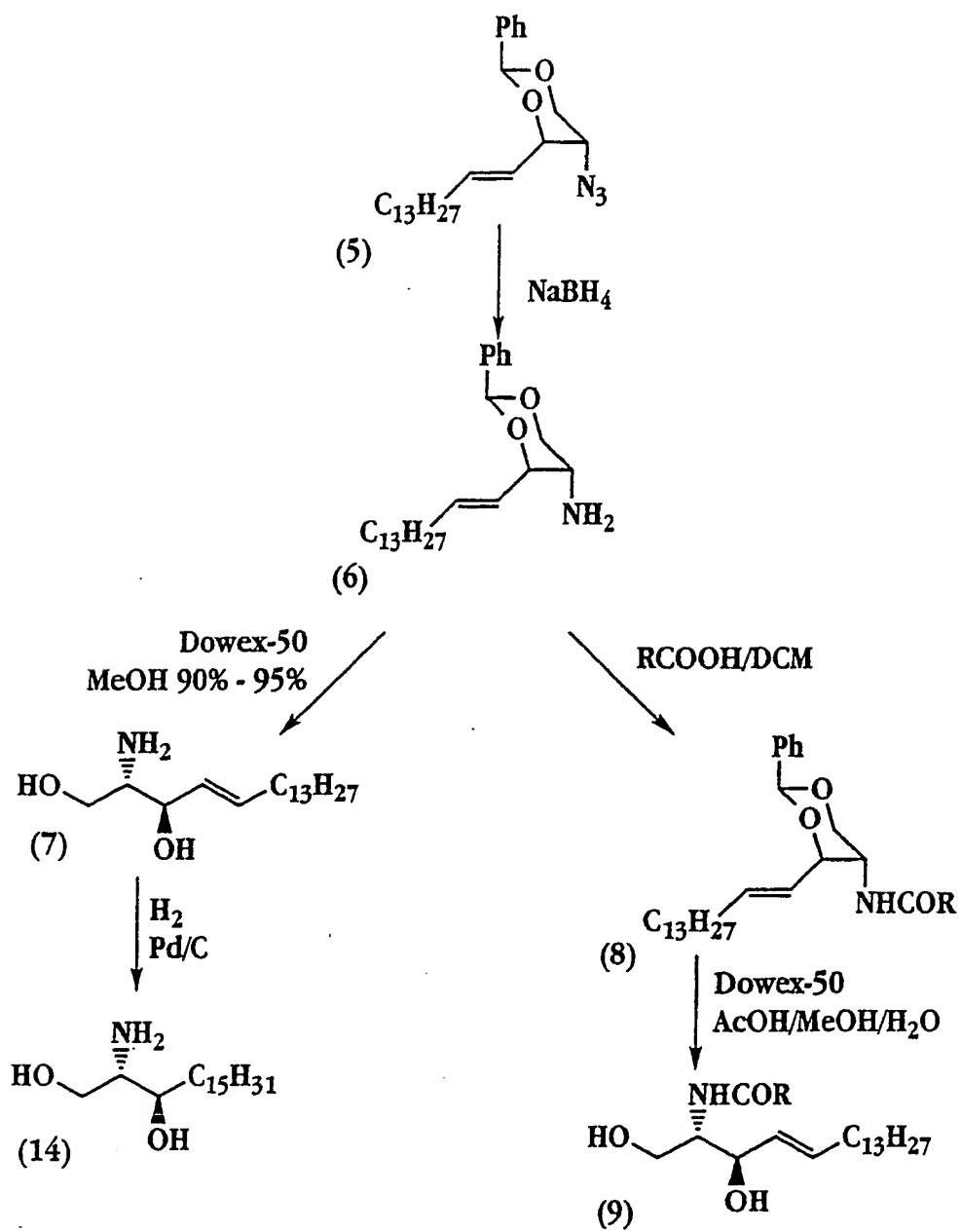


Fig. 2

3/3

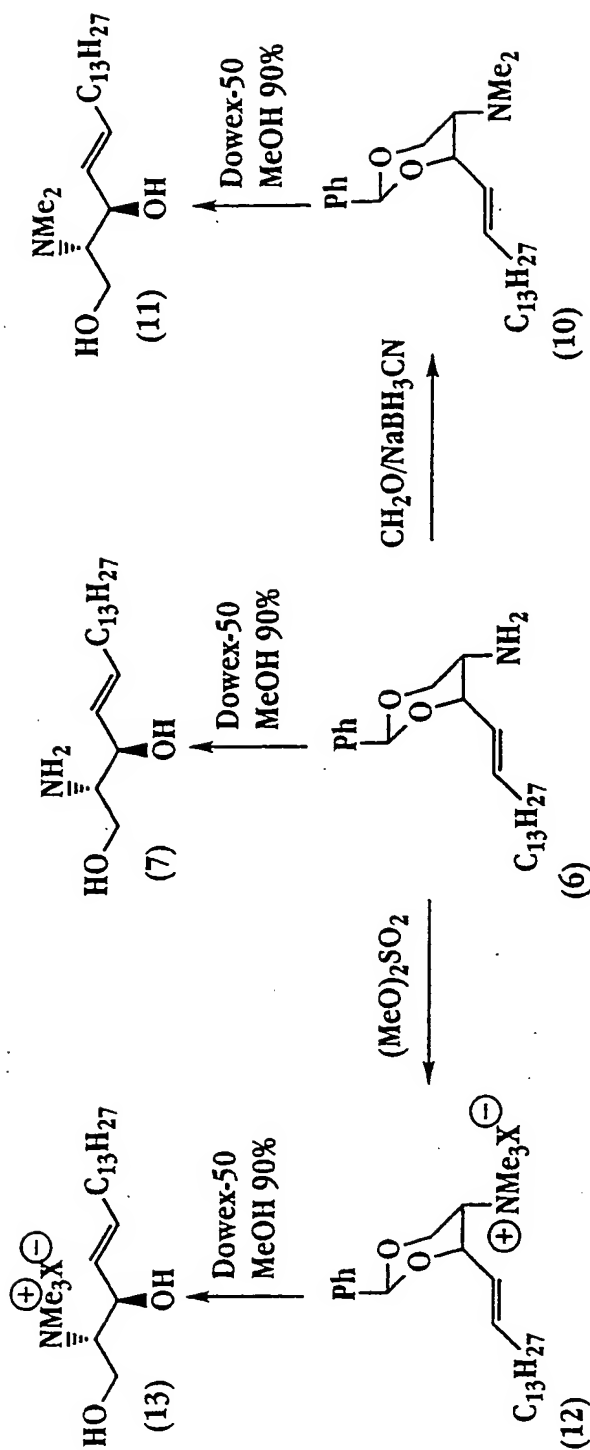


Fig. 3

# INTERNATIONAL SEARCH REPORT

In. tional Application No

PCT/IL 00/00264

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07H9/04 C07C213/00 C07C231/18

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07H C07C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, CHEM ABS Data

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	K. OHASHI ET AL.: "Synthesis of D-erythro-1-deoxydihydroceramide-1-sulfonic acid" TETRAHEDRON LETTERS, vol. 29, 1988, pages 1185-1188, XP002141910 the whole document	1-5
A	US 4 937 328 A (SCHMIDT RICHARD R ET AL) 26 June 1990 (1990-06-26) cited in the application the whole document	1-5

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

5 July 2000

Date of mailing of the international search report

25/07/2000

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl.  
Fax: (+31-70) 340-3016

Authorized officer

de Nooy, A

# INTERNATIONAL SEARCH REPORT -

In International Application No

PCT/IL 00/00264

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>E.G. GROS, V. DEULOFEU: "Reaction of ammonia with some acetylated and benzoylated monosaccharides. IX. The migration of benzoyl groups in the ammonolysis of 1,2,3,4,6-penta-O-benzoyl-D-galactoses" J. ORG. CHEM., vol. 29, 1964, pages 3647-3654, XP002141911 cited in the application page 3652, left-hand column, paragraph 5</p> <p>-----</p>	1-5

1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

page 2 of 2

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/IL 00/00264

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 4937328 A	26-06-1990	AT 71104 T	15-01-1992
		AU 603773 B	29-11-1990
		AU 6108386 A	19-02-1987
		CA 1267891 A	17-04-1990
		DE 3683214 A	13-02-1992
		DK 382486 A,B,	14-02-1987
		EP 0212400 A	04-03-1987
		ES 2001208 A	01-05-1988
		FI 863272 A,B,	14-02-1987
		HU 197916 B	28-06-1989
		NO 863251 A,B,	16-02-1987
		YU 142886 A	30-06-1988
		DD 261165 A	19-10-1988
		JP 62039597 A	20-02-1987
		ZA 8606023 A	25-03-1987

Form PCT/ISA/210 (patent family annex) (July 1992)

**This Page is Inserted by IFW Indexing and Scanning  
Operations and is not part of the Official Record**

**BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☒ FADED TEXT OR DRAWING
- ☒ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☒ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☒ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: \_\_\_\_\_

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.**

**THIS PAGE BLANK (USPTO)**